Discussion. The ability of zinc sulphate pretreatment to produce dose-related decreases in the incidence of stress-induced gastric lesions, as well as the gastric secretory volume and total acid output in nonstressed or in stressed rats, confirms previous observations and conclusions[3]. However, the associated dose-related changes in stomach wall mucus suggest a relationship between gastric wall mucus and stress ulcer protection by zinc sulphate. Since the adopted method[6] measures mucus in the superficial secretory layer of the gastric mucosa, it is reasonable to deduce that an increase in mucus adhering to the gastric mucosa would provide greater protection against the acidity and peptic activity of gastric juice[2]. The high incidence of stress ulcers associated with a significant decrease in gastric wall mucus, and the reversal of these parameters by zinc sulphate do indeed support this deduction.

Zinc sulphate has already been shown to reduce the severity of gastric ulcers in man[7-9]; thus it is conceivable that its mucus-increasing effect could contribute to increased mucosal resistance to ulceration. The present observations indicate an action on the stomach following absorption from a parenteral site of administration, whereas clinical investigations[7-9], using the oral route, only suggest this possibility. It is unlikely that zinc sulphate produces its effects through a toxic action[10, 11].

The mechanism through which zinc sulphate exerts its mucus-increasing effect is unclear, but 3 possibilities may be speculated upon; inhibition of gastric histamine release[10] may increase mucus formation[2], the sulphate moiety of the preparation may influence chondroitin sulphate synthesis[2, 12], or zinc itself may stimulate mucus formation.

7 P. M. Fraser, R. Doll, M. J. S. Langman, J. J. Misiewicz and H. H. Shawdon, Gut *13*, 459 (1972).
8 D. J. Frommer, Med. J. Aust. *2*, 793 (1975).
9 K. B. Orr, Med. J. Aust. *1*, 244 (1976).
10 C. H. Cho and C. W. Ogle, Eur. J. Pharmac. *43*, 315 (1977).
11 C. H. Cho, C. W. Ogle and S. Dai, IRCS Med. Sci. *4*, 469 (1976).
12 H. Boström, J. biol. Chem. *196*, 477 (1952).

Chemical protection of mouse spermatocytes against gamma-rays with 2-mercaptopropionylglycine

P. Uma Devi and B. R. Saharan

Radiation Biology Laboratory, Department of Zoology, University of Rajasthan, Jaipur 302004 (India), 18 April 1977

Summary. The radiosensitivity of primary spermatocytes in pachytene stage was estimated by counting the number of spermatids in the testes of control and MPG-treated mouse after exposure to 500, 1000 and 1500 R of Co^{60} gamma-rays. For this purpose, control and MPG-treated mice were killed 5 days after irradiation and countings of spermatids was made in stages I and II of the tubules. It has been observed that, although there was a death of primary spermatocytes in irradiated MPG-protected groups, quantitatively significant protection was afforded by this drug at all the 3 dose-levels studied.

Since the radioprotective properties of MEA[1] and AET[2] became known, many studies have been carried out to study the effect of sulfhydryl compounds in modifying radiation injury, especially in the mammalian germ cells. In 1953, Kaplan and Lyon[3], published their first report on experiments in which 2-mercaptoethylamine (MEA) was used in an attempt to protect the hereditary material against ionizing radiation. But the later works in this field were mainly concerned with clarifying the ability of different substances to prevent radiation-induced changes in the individual's reproductive mechanisms rather than concentrating on the purely genetic consequences of irradiation. However, recently, many unsuccessful attempts have been made at chemical protection from radiation related to genetic damages[4, 5]. Leonard and Deknudt[6] have presented evidence that some radioprotective agents, administered simultaneously, gave a favourable effect with regard to translocation resulting from spermatogonial X-irradiation in the mouse. A combination of ATP-AET-serotonin had provided highly significant efficiency in protecting the mouse spermatogonia against 300 R of X-rays[7]. Most of the study up to date used chemical protectors (cysteine, cysteamine and AET) to modify injury in mammalian germ cells against X-rays. Present study deals with the radioprotective action of MPG on the primary spermatocytes of mouse testes against various doses of gamma-rays.

Material and methods. Swiss albino male mice from an inbred colony, 6–8 weeks old, and weighing about 24 g, were used in the experiments. 3 sets of experiments were conducted, each using a different irradiation dose. Each set contained an experimental (MPG-treated) and a control group with equal number of animals. The experimental animals in each set were injected with 20 mg/kg b.wt of MPG (2-mercaptopropionylglycine received from Santen Pharmaceutical, Osaka, Japan, dissolved in distilled water with PH adjusted to 6.4 with 0.1 N NaOH before use) i.p. and the control group received an equal volume of distilled water in the same manner. After treatment, the animals of the 3 sets were exposed to a Co^{60} gamma-source to give a total dose of 500, 1000 and 1500 R respectively at the dose rate of 25 R/min. 3 mice of each treatment (control and drug-treated) were killed at 5 days after post-irradiation, and testes were removed. The testes of each mouse was fixed in Bouin's fluid and 6 μm sections were stained with Harris hematoxylin-eosin and PAS-hematoxylin for qualitative and quantitative studies respectively. Number of spermatids were counted in I and II stage, in carefully selected median cross-sections of the tubules from each mouse testes. Spermatids were counted only if the greater part of the nucleus was included in the sections (intensity of staining and presence

1 Z. M. Bacq, G. Deschamps, P. Fisher, A. Herve, H. Le Bihan, J. Le Comat, M. Pirole and P. Rayet, Science *117*, 633 (1953).
2 D. G. Doherty, W. T. Burnett, Jr, and R. Shapira, Radiat. Res. *7*, 13 (1957).
3 W. D. Kaplan and M. F. Lyon, Science *118*, 777 (153).
4 A. Leonard and J. R. Maisin, Radiat. Res. *23*, 53 (1964).
5 A. Leonard and G. H. Deknudt, Radiat. Res. *50*, 120 (1972).
6 A. Leonard and G. H. Deknudt, Strahlentherapie *145*, 174 (1973).
7 D. K. Benova and I. A. Baev, Int. J. Radiat. Biol. *26*, 47 (1974).

of definite membrane were the criteria used). Only those cells with obvious degenerative changes were considered necrotic. Counts were corrected by Aberchrombie's method to eliminate errors due to variation in nuclear diameter.

Results and discussion. The variations in the population dynamics of spermatids in control and MPG-treated mice 5 days after exposure to 500, 1000 and 1500 R are presented in the table. At 5-day-interval, the numbers of spermatids in control mice are found to be 60.50, 43.00 and 30.00 at 500, 1000 and 1500 R respectively whereas in MPG-treated mice the counts observed are 84.00, 65.45 and 50.00 respectively and these data are statistically highly significant. Our observations on the number of spermatids clearly show that MPG largely protects the radiation-induced lesions produced in the primary spermatocytes. Our data are highly significant as studied up to 1500 R dose. Histopathological studies at 500 R, reveal that a higher number of necrotic spermatocytes was observed in diakinesis metaphase 1 in nonprotected groups, as compared with control mice[8]. Irradiation damage of primary spermatocytes remain latent until the cell enters meiotic metaphase and anaphase. Many abnormal figures typical of chromosomal aberrations thus occur, and nuclei of resulting spermatids show abnormal size variations (giant cells). These giant spermatids are formed by spermatocytes irradiated in pacyhtene, and are greater in number in nonprotected mice with increased doses of irradiation of 1000 R and 1500 R (unpublished data). There is considerable evidence that DNA is the major target of ionizing radiations in the living cells[9,10]. Significant chemical protection of DNA-molecule provide significant protection against radiation death[5]. It is not known whether these chemical protectors protect DNA directly or indirectly (by preventing radiation-induced release of DNA-destructive enzymes) or protect against radiation-induced destruction of DNA repair system.

Effect of MPG on the survival of spermatids* 5 days after whole body exposure of Swiss albino mice to different doses of gamma-radiation**

Doses		Total No. of tubule cross-section counted	Total number of spermatids	Mean number of spermatids ± SE
500 R	Control	20	1210	60.50 ± 3.10
	Drug-treated	20	1560	84.00 ± 2.50
				$p < 0.001$
1000 R	Control	20	871	43.00 ± 2.00
	Drug-treated	20	1389	65.45 ± 3.30
				$p < 0.001$
1500 R	Control	20	560	32.25 ± 2.00
	Drug-treated	20	1005	50.00 ± 2.80
				$p < 0.001$

* No. of spermatids in normal nonirradiated mouse testis = 104 ± 3 per tubule cross-section.

** Sensitivity of primary spermatocytes scored by counting the number of spermatids in stage I and II of tubules, 5 days after post-irradiation.

The most plausible explanation with regard to the mechanism of protection relates to the possibility that some –SH compounds partially or completely inhibit the deleterious action of the free radicals normally found in X-irradiated tissue; or they may even modify the nature of these radicals in such a manner as to render them less harmful[11–13]. It is also thought possible that the effective –SH carrying compounds may combine temporarily with some radiosensitive enzymes, making them radio-resistant[11]. The exact mechanism of MPG protection is not known; however, these explanations may also apply to our findings.

The present results indicate that MPG very likely protects the chromosomes in the pachytene of meiosis, thus allowing more spermatocytes to survive and become normal spermatids. This is possible either by reducing the number of radiation-induced chromosome breaks or by causing a restitution of the broken chromosomes. Further, investigations with a view to understanding this mechanism is in progress in our laboratory.

8 B. R. Saharan and P. Uma Devi, J. Radiat. Res. *18*, 308 (1977).
9 H. S. Johannes, Int. J. Radiat. Biol. *16*, 377 (1969).
10 F. Fremuth and M. Sadilkova, Folia biol. *22*, 196 (1976).
11 A. Hervey and Z. M. Bacq, J. belge Radiol. *35*, 524 (1952).
12 E. S. G. Barron and S. R. Dickman, J. gen. physiol. *32*, 595 (1949).
13 L. O. Jacobson, Cancer Res. *12*, 595 (1952).

Histochemical studies on two types of cells containing catecholamines in sympathetic ganglia of the bullfrog

H. Kojima, S. Anraku, K. Onogi[1] and R. Ito[1,2]

Institute of Brain Diseases, Kurume University School of Medicine, Kurume 830 (Japan), 13 June 1977

Summary. The excitation/emission spectra maxima obtained from orange fluorescing small cell clusters and greenish yellow fluorescing ganglion cell bodies were at 400–410/490–560 nm and at 400–410/470–500 nm, respectively.

Azuma et al.[3] reported from a viewpoint of biochemical determination that the sympathetic ganglion chain of the bullfrog contained catecholamines, such as noradrenaline and adrenaline, in a relatively large quantity.

On the other hand, Kojima[4], using formaldehyde-induced fluorescence microscopy[5], mentioned that there were 2 types of cells, namely the small cell clusters and the ordinary ganglion cell bodies showing specific fluorescences in the sympathetic ganglia of the bullfrog. According to his work, it was suggested that the small cell clusters and the ganglion cell bodies would contain mainly a primary catecholamine (dopamine or noradrenaline) and a secondary catecholamine (adrenaline), respectively.

A further experiment was attempted to demonstrate the cellular catecholamines in bullfrog sympathetic ganglia by means of fluorescence microspectrophotometry[6] and